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Imidazolopyrazinones as Potential Antioxidants

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Abstract—A series of imidazolopyrazinones **3**, substituted at C-2, and C-2/C-6, has been prepared. The compounds behaved as quenchers of superoxide anion. The more active compounds are structurally related to coelenterazine, a natural substrate of marine bioluminescence. Theoretical parameters based on Hartree–Fock instabilities have been examined.

Partially reduced derivatives of oxygen, which are produced in aerobic organisms as part of normal physiological and metabolic processes, are toxic species since they can oxidize numerous biomolecules leading to tissue injury and cell death.[1] Such reactive oxygen species (ROS), produced in excessive concentrations or in wrong locations, cause an oxidative stress associated with a variety of disease states in humans.[2–4] Thus, when the natural protective systems towards ROS is running over, exogeneous antioxidative compounds have to be delivered. The research of new antioxidants as potential drugs is an active field of medicinal chemistry.[5–7] The compounds usually involve *N*-heterocycle and/or phenol moieties as radical scavengers.

Recently, we demonstrated that coelenterazine (CLZ; Scheme 1, Table 1) shows high antioxidative properties in cells submitted to oxidative stress induced by *t*-butyl hydroperoxide, and inhibits the oxidation of linoleate initiated by azoperoxyl radicals.[8–10] Coelenterazine is an imidazolopyrazinone derivative isolated from marine organisms; this natural compound is the chromophoric ligand of a calcium-sensitive photoprotein called aequorin.[11–13] The molecular mechanism of CLZ bioluminescence and chemiluminescence is still a subject of investigations:[14,15] the catalyzed oxidation of CLZ produces carbon dioxide and coelenteramide in an excited state which deactivates by emission of light.

The sensitivity of CLZ towards oxygen and ROS led us to consider this heterocyclic system as a potential lead in medicinal chemistry for the discovery of new antioxidants.[16] In this paper, a series of simple imidazolopyrazinones, structurally related to CLZ, has been prepared and evaluated in a standard test towards superoxide anion, in view to assess the interest of this class of compounds. Theoretical evaluation also suggested a high antioxidative potential for imidazolopyrazinone compounds.

Imidazolopyrazinones **3a**–**l** (Scheme 1, Table 1) were readily obtained by condensing 2-aminopyrazines **1** with α-keto-aldehydes (or the derived acetals) **2**, according to known procedures.[16–20] 5-Aryl-2-aminopyrazines ($R^1 \neq H$) resulted from a Suzuki-like coupling[21] of 5-bromo-2-aminopyrazine[22] with arylboronic acids (phenylboronic acid and 4-methoxyphenylboronic acid). The deprotection of 5-(4-methoxyphenyl)-2-aminopyrazine into 5-(4-hydroxyphenyl)-2-aminopyrazine was realized with sodium ethanethiolate in DMF at 100 °C.[23] The diethyl acetal of benzyl glyoxal ($R^3 = PhCH_2$) was prepared by substitution of 1-(diethoxyacetyl) piperidine with benzylmagnesium bromide.[24]

Compounds **3**, isolated as the hydrochloride salts,[25] were characterized by NMR spectroscopy. Since no

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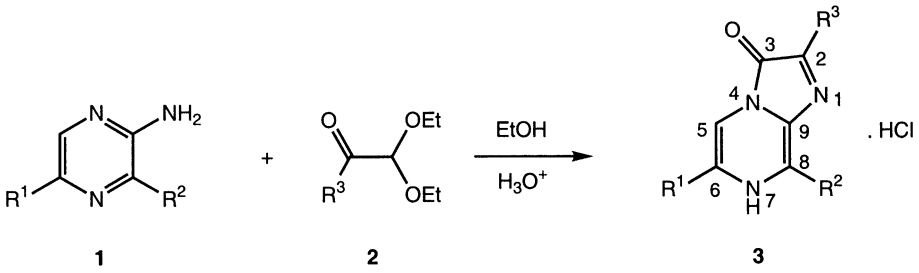

Scheme 1.

complete data for imidazolopyrazinones[26] have been found in literature, we collected the spectroscopic values of **3a**–**l** in Tables 2 and 3 (spectra recorded respectively at 500 and 125 MHz). Typically, in the ^{1}H spectra (Table 2), H-5 appeared at 8.2–8.5 δ when R^3 is an alkyl group ($R^3 = CH_3$, CH_2Ph), and at 7.8–8.1 δ when R^3 is phenyl. Similarly, H-8 was slightly shielded when R^3 is phenyl (8.3–8.5 δ) comparatively to the chemical shift found for this proton when R^3 is an alkyl group (8.6–8.9 δ). The ^{13}C spectra (Table 3) showed the signal attributed to the carbonyl function (C-3) around 148 δ when R^3 is phenyl, and at 136–142 δ when R^3 is an alkyl group ($R^3 = CH_3$, CH_2Ph). This is consistent with previous data obtained by X-ray diffraction analysis of crystals:[16] 2-phenylimidazolopyrazinone **3b** appeared in the ketone form, while 2-methylimidazolopyrazinone **3a** was stabilized in the enol tautomeric form. In structures **3d**–**l**, the chemical shifts of C-2 and C-9, two atoms making part of the conjugated system which extends from N-7 to C-3,[16] are very similar. On the other hand, C-6 and C-8 appear to be influenced by the nature (aryl or alkyl) of the substituent R^3.

The chemical reactivity of CLZ towards ROS has been correlated to the rate constant of its reaction with superoxide anion using the hypoxanthine–xanthine oxidase system.[27,28] The same test was used for the evaluation of the synthetic imidazolopyrazinones in the presence of MeO–CLZ (Scheme 1, Table 1) as the competitor. The rate constant of this luminescent reference[16] has been previously determined by using Trolox^R, a water-soluble derivative of vitamin E,[29] as the known competitor (Table 4). Thus, compounds **3a**–**l** were reacted with $O_2^{-\bullet}$ in the presence of MeO–CLZ in different concentrations;[30] the intensities of light emission were measured at 380 nm, and the reaction rate constants were calculated from the following equation:[31–33]

$$I_o/I_c = 1 + k_c/k_i \times [\text{MeO}-\text{CLZ}]/[\mathbf{3}]$$

where I_o = luminescence measured without MeO–CLZ, I_c = luminescence measured in the presence of MeO–CLZ (competitor), k_c = rate constant of MeO–CLZ (competitor), k_i = rate constant of the tested compound **3**, [MeO–CLZ] = concentration of competitor and [**3**] = concentration of tested compound.

Table 1. Imidazolopyrazinone derivatives

Compd	R^1	R^2	R^3	Ref
CLZ	*p*-HO-Ph	CH_2Ph	CH_2-Ph-*p*-OH	17–19
MeO–CLZ	*p*-MeO-Ph	CH_2Ph	CH_2-Ph-*p*-OH	8,10
3a	H	H	Me	16
3b	H	H	Ph	16
3c	H	H	CH_2Ph	20
3d	Ph	H	Me	20
3e	Ph	H	Ph	20
3f	Ph	H	CH_2Ph	20
3g	*p*-MeO-Ph	H	Me	20
3h	*p*-MeO-Ph	H	Ph	20
3i	*p*-MeO-Ph	H	CH_2Ph	20
3j	*p*-HO-Ph	H	Me	20
3k	*p*-HO-Ph	H	Ph	20
3l	*p*-HO-Ph	H	CH_2Ph	20

Table 2. ^{1}H NMR data (δ)

Compd	Solvent	H-5	H-6	H-8	*J* (Hz)
3a	CD_3OD	8.09	7.65	8.78	$^3J_{5-6} = 5.5$; $^5J_{5-8} = 1.1$
	DMSO-d_6	8.30	7.73	8.99	$^4J_{6-8} = 0.5$
3b	CD_3OD	8.29	7.70	8.86	$^3J_{5-6} = 5.5$; $^5J_{5-8} = 0.8$
	DMSO-d_6	7.87	7.22	8.47	$^4J_{6-8} = 0.5$
3c	CD_3OD	8.19	7.70	8.77	$^3J_{5-6} = 5.5$; $^5J_{5-8} = 0.9$
					$^4J_{6-8} = 0.5$
3d	CD_3OD	8.30	—	8.76	$^5J_{5-8} = 1.1$
3e	DMSO-d_6	8.15	—	8.44	
3f	CD_3OD	8.24	—	8.61	$^5J_{5-8} = 0.8$
3g	CD_3OD	8.41	—	8.88	$^5J_{5-8} = 1.1$
3h	DMSO-d_6	7.89	—	8.30	
3i	CD_3OD	8.57	—	8.96	$^5J_{5-8} = 0.9$
3j	DMSO-d_6	8.48	—	8.90	
3k	DMSO-d_6	7.88	—	8.33	
3l	CD_3OD	8.42	—	8.84	$^5J_{5-8} = 0.9$

Table 3. ^{13}C NMR data (δ)

Compd	Solvent	C-2	C-3	C-5	C-6	C-8	C-9
3a	CD_3OD	135.9	142.8	116.5	122.4	133.6	130.3
	DMSO-d_6	137.4	142.9	114.4	119.1	130.1	128.8
3b	CD_3OD	139.0	148.2	113.4	116.1	127.1	129.0
	DMSO-d_6	139.4	149.9	112.5	115.5	125.7	129.1
3c	CD_3OD	140.3	144.5	116.2	121.2	132.6	130.7
3d	CD_3OD	123.9	139.9	112.4	141.2	135.8	128.5
3e	DMSO-d_6	128.7	148.0	109.5	129.2	128.0	129.3
3f	CD_3OD	124.3	141.4	112.5	141.2	134.8	129.3
3g	CD_3OD	126.9	140.0	111.2	141.0	134.7	128.8
3h	DMSO-d_6	128.7	148.7	108.3	129.5	127.2	129.3
3i	CD_3OD	126.2	140.5	111.4	141.0	133.4	129.0
3j	DMSO-d_6	127.0	137.5	109.6	139.2	133.6	127.0
3k	DMSO-d_6	128.6	147.8	108.1	129.2	127.6	129.3
3l	CD_3OD	127.4	136.5	110.7	141.4	135.0	129.5

The results, summarized in Table 4, clearly showed that all the tested imidazolopyrazinones are more active than Trolox^R; however, two compounds (**3e** and **3h**) could not be evaluated because they were not totally soluble in the required concentrations. The presence of an aryl substituent R^1 at position C-6 increased the activity, comparatively to the C-6 unsubstituted series (compounds **3d**, **3g**, and **3j**). The substituent R^3 at position C-2 appeared to exercise a moderate influence. Accordingly, the *p*-hydroxybenzyl substituent found in the natural CLZ is not absolutely required. Compared to the natural derivatives (CLZ and MeO–CLZ) possessing a benzyl substituent (R^2) at position C-8, the synthetic derivatives **3** devoid of such a substitution are 2 or 3 times more active. Thus, simple imidazolopyrazinones, more easily synthesized than CLZ, can be considered as potential antioxidants.

Theoretical parameters have been recently defined to characterize antioxidants and to predict antioxidative activities. Testa et al.[34,35] considered ΔH_{ox} (relative adiabatic oxidation potential) and the shape of the SOMO (singly occupied molecular orbital) as the quantum chemical descriptors. More recently, Haemers et al.[36] correlated antioxidant activity with radical stabilization properties. These parameters are derived from the analysis of the radical form obtained by elimination of one electron. In the present study, an analysis of the propension of the neutral compounds **3** with all their paired electrons to generate radicalar structures is concerned. This approach is based on the investigation of Hartree–Fock instabilities. By its fused five-membered ring, the imidazolopyrazinone compounds break down the aromaticity and only one Kekulé form can be drawn. This feature has been previously pointed out as a source of the wave function instability.[37] It is related to the vicinity of a triplet electronic state near the fundamental singlet state. Imidazole wave function is stable and that of pyrazine presents the same weak instability as benzene and pyridine; but the association of the two fragments leads to a significant instability. This one can be seen as a propensity of the molecules to present a local biradicalar character which could directly be related to their reactivity towards superoxide anion.

Table 4. Reaction rate constants with O_2^-

Compd	$k_i \times 10^4$ (M^{-1} s^{-1})	k_{rel}[a]
Trolox	1.7[28]	1
MeO–CLZ	9.6±0.1[15]	5.6
3a	6.0±0.1	3.5
3b	8.4±0.6	4.9
3c	2.8±0.3	1.6
3d	17±0.2	10.0
3e	n.d.[b]	—
3f	6.5±0.1	3.8
3g	28±0.1	16.5
3h	n.d.	—
3i	16±0.3	9.4
3j	21±0.4	12.3
3k	10.5±0.3	6.2
3l	3.3±0.2	1.9
CLZ	12±0.1[16]	7.1

[a] $k_{rel} = k_i/k_{Trolox}$.
[b] n.d., not determined for solubility reasons.

The Hartree–Fock instabilities[38] of compounds **3** are summarized in Table 5. At the singlet state structure, the optimization of the wave function gives rise to a stabilization energy (delta-stable). From this result, a complete geometry re-optimization generates the relative energy values at the UHF level (UHF/UHF) in which alpha and beta electrons occupy different spatial molecular orbitals. The more the molecule is substituted by aromatic fragments, the more the stabilization energy is high. This feature is nicely correlated to the antioxidant activity for the four compounds bearing a methyl substituent at position C-2 ($R^3 = CH_3$), namely **3a**, **3d**, **3j**, and **3g**. With a benzyl substituent in that position ($R^3 = CH_2$–Ph), additional steric effects could explain the less clear relation with activity, except between **3c** and **3i**. Figure 1 shows the spin density of the reference compound **3** ($R^1 = R^2 = R^3 = H$): the isocontour of spin density at 0.005 AU (e/A**^{3}) displays the alternation of positive and negative clouds. The break induced by the substitution is illustrated with compound **3j** (Fig. 1).[39] For both molecules, the highest spin density is located on the carbon atoms C-2 and C-8.

Thus, imidazolopyrazinones represent a valuable class of new antioxidants; an aryl substituent R^1 at position

Table 5. Theoretical parameters

Compd	Delta-E stable	Delta-E UHF/UHF	Stabilization
3a	16.29	27.89	11.60
3b	31.18	46.48	15.30
3c	25.00	39.72	14.72
3d	28.37	44.01	15.64
3f	36.96	54.75	17.79
3g	27.35	42.78	15.43
3i	35.60	53.92	18.32
3j	27.42	42.87	15.45
3l	35.52	52.87	17.35
Reference[a]	17.59	30.36	12.77

[a] Reference = compd **3** with $R^1 = R^2 = R^3 = H$.

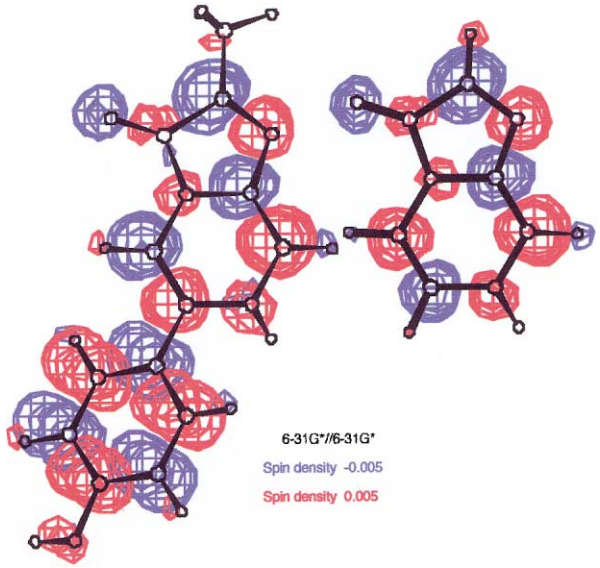

Figure 1.

C-6, in particular a phenol moiety, reinforces this property, while the R^3 substituent at position C-2 seems to play a moderate role. The C-8 position, substituted with a benzyl group ($R^2 = Bz$) in the natural CLZ but unsubstituted in the synthetic derivatives **3a–l** ($R^2 = H$), could be used for the anchorage of molecular fragments susceptible to improve the biodisponibility.

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25. Typical procedures for the preparation of compounds **3**: Method A ($R^3 = Me$, Ph): to a 0.5 M solution of **1** (1 equiv) and methyl- or phenylglyoxal **2** (1.5 equiv) in ethanol, was added aqueous HCl (37%, 3.6 equiv). The mixture was heated under argon atmosphere for 4 h at 80 °C, then concentrated in vacuum. The residue was dissolved in cold methanol and left overnight at −18 °C to crystallize. The solid was filtered off and washed several times with cold methanol, ethyl acetate and ether. Method B ($R^3 = CH_2Ph$): to a 0.25 M solution of **1** (1 equiv) and benzylglyoxal **2** (diethyl acetal, 1.3 equiv) in dioxane–water (2:1, v/v), was added aqueous HCl (37%, 10 equiv). The mixture was heated under argon atmosphere for 4 h at reflux, then concentrated in vacuum. The residue, dissolved in methanol, was precipitated by addition of cold ether.
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30. Typical procedure for the reaction with superoxide anion: hypoxanthine (HX; first dissolved in 1 N NaOH), xanthine oxidase (XOD), Trolox (6-OH-2,5,7,8-tetramethylchroman-2-carboxylic acid; first solubilized in DMSO), and albumin were purchased from Sigma-Aldrich. All the solutions were made at 25 °C in 50 mM Tris–HCl buffer (pH 7.8) containing EDTA (0.1 mM). The final concentrations of HX and XOD were 500 μM and 8.25 U/L, respectively. Albumin was added at a final concentration of 15 mg/L to minimize the inactivation of XOD. During the stationary phase of the reaction, the light yields [relative luminescence units (RLU)] were recorded for 200 s, in 96-well plates, with a Microlumat LB96P6 luminometer (Berthold Inc., Wildbad, Germany). Each well contains a total volume of 200 μL after addition of HX, that is 40 μL of the competitor (25 μM), 40 μL of **3** (from 25 to 250 μM), 15 μL of XOD, 55 μL of buffer, and 50 μL of HX. Background chemiluminescence (before HX addition) was subtracted from the luminescence signal. Competitive quenching experiments towards $O_2^{-\bullet}$ were performed between luminescent compounds (CLZ, MeO–CLZ; 5 μM) and increasing Trolox^R concentrations (from 0 to 60 μM). Rate constants of non-luminescent compounds (**3a–l**) towards $O_2^{-\bullet}$ were obtained by competition between them and MeO–CLZ. Plotting [MeO–CLZ]/[**3**] versus the I_o/I_c values allows the determination of k_c/k_i from the slope of the linear relationship linking these parameters. Each experiment was performed six times.
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38. Computational tool: all the geometry optimizations and instability calculations have been performed at the ab initio level using the MINI-1′ basis set.[40,41] As pointed out in the

study of Hartree–Fock instabilities occurring in benzimidazole derivatives,[42] MINI-1′ provides results well correlated with those derived from extended basis sets.
39. Computational tool: the calculations have been performed at the ab initio level using the 6-31G* basis set (self-consistent molecular orbital method 25: supplementary functions for gaussian basis sets)[43] and the numerical procedures available in Gaussian 94 (revision E.2).[44]
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